INVESTIGATIONS OF TWO-SPORED FORMS IN THE GENUS MYCENA

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INVESTIGATIONS OF TWO-SPORED FORMS IN THE GENUS MYCENA [1]

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(WITH PLATES 34–38)

INTRODUCTION

It has long been known that within the genus *Mycena* there exists an unusually large number of forms bearing basidia which produce two instead of the usual four spores. In recent years even more variation has been noticed (Oort, **19**). In general the use of the two-spored basidium as a character of taxonomic importance in the Agaricaceae has been questioned. There still seems to be a difference of opinion, however. Lange (**13**, **14**) by using this character in keying out species of *Mycena* and *Omphalia,* and in describing a two-spored species of *Tricholoma* has given it considerable weight. Kauffman in his unpublished manuscript on *Mycena* had also placed considerable emphasis on the two-spored character, chiefly it seems, because in *Mycena* an increase in spore size usually accompanies the change from the four- to the two-spored basidium.

In comparatively recent times considerable emphasis has been given to cytological and genetical studies of two-spored forms in the Agaricaceae and related families. The investigations of Bauch (**1**, **2**), Buhr (**4**), Fries (**5**, **6**), Harper (**7**), Juel (**8**), Kühner (**10**, **11**, **12**), Lewis (**16**), and Sass (**20**, **21**) have given much information concerning the types of nuclear behavior in scattered species throughout most of the families of higher Basidiomycetes. In most species with two-spored basidia it has been found that there is a fusion of two primary nuclei in the basidium and that in the Agaricaceae, as a rule, two divisions follow before the spores mature. In the Clavariaceae three divisions are usually found. In the Agaricaceae, depending on the species, all of the four nuclei

[1] Papers from the Department of Botany and the Herbarium of the University of Michigan, No. 455.

may migrate or two may degenerate in the basidium. A few forms have been described which are parthenogenetic. In the latter the young basidium contains only a single nucleus.

Since no intensive study had been made involving both those species which are known to have four- and two-spored forms, and those isolated species in which only two-spored basidia are known, it seemed very desirable to investigate as many of both types as could be found in a single genus in order to furnish, if possible, a better understanding of the position of two-spored forms in the classification of agarics, and in addition to obtain information concerning the nuclear history in a large number of closely related variant forms. The genus *Mycena* seemed to be a particularly favorable group of species for such an investigation because of the relative abundance of material, and because of the fact that both of the types of nuclear history mentioned above had previously been found among its species.

The writer wishes to express his appreciation to Professor E. B. Mains for advice and guidance during the course of the present investigation, and to Professor W. R. Taylor for his interest and helpful suggestions in the cytological studies.

MATERIALS AND METHODS

Since few cytological investigations have previously been made on species of *Mycena, M. alcalina* Fries, *M. Atkinsoni* House, *M. hemisphaerica* Peck, *M. haematopoda* Fries, *M. leptocephala* Fries, *M. murina* Murr., *M. sanguinolenta* Alb. & Schw., *M. stannea* Fries and *M. viscosa* Maire were studied in order to establish the usual type of nuclear behavior. The following nineteen variant forms were investigated: *Mycena alcalina* Fries, *M. capillaris* Fries, *M. citrinomarginata* Gillet, *M. clavicularis* Fries, **M. cholea** sp. nov.,[2] *M. graveolens* Kauff. & Smith, *M. immaculata* Peck, *M.*

[2] **Mycena cholea** sp. nov.—Statura Mycenae vitilis; pileus 2–12 mm. latus, obtuse conicus demum campanulate expansus, fuscus vel bruneolus, expallescens, striatus, non pruinosus; lamellae subconfertae, adnatae, albidae interdum pallide sordidae; caro incisa demum incarnata, sapore quininae simili, praecipue in stipite succosus, succo lacteo non copioso; stipes (1) 3–6 (10) cm. longus, 1 mm. crassus, fuscus demum pallidus; sporae 9–11 (12) × 6–7.5 (8) μ, ovoideae; basidia bispora; cystidia numerosa, apice cuspidata, 55–80 × 10–14 μ. Specimen typicum in Herb. Mich. conservatum, A. H. Smith n. 32–545, prope Ann Arbor, Mich. Oct. 8, 1932.

lasiosperma Bres., *M. dissiliens* Fries, *M. leptocephala* Fries, *M. margaritispora* Lange, *M. megaspora* Kauff., *M. metata* Fries, *M. mirata* Peck, *M. polygramma* Fries var. *albida* Kauff., *M. roseipallens* Murr., *M. rubromarginata* var. **Laricis** var. nov.,[3] *M. viscosa* Maire, and *M. vitilis* Fries.

The cytological studies have all been made from fruit-bodies collected in the vicinity of Ann Arbor. Because many of the species in this genus are very small and delicate it is desirable to kill and fix them for cytological study in the field when they are collected. In killing such material it is necessary to remove the air from between the lamellae in order to obtain the best results. It was found that by reversing the leather on the plunger in an ordinary tire-pump, and fitting the rubber tube with the proper connections, a very effective apparatus could be made. It gave rapid evacuation without damage to the delicate lamellae, and could conveniently be carried into the field.

The most satisfactory killing solutions were those of Flemming in various dilutions, and the following two known as Allen's modification of Bouin's solution.

1. After La Cour (15)	
Picric acid, sat. aq. sol.	75.0 cc.
Formalin	25.0 cc.
Acetic acid (Glacial)	5.0 cc.
Urea	2.0 gms.
Chromic acid	1.5 gms.
2. Picric acid, sat. aq. sol.	75.0 cc.
Formalin	25.0 cc.
Acetic acid (Glacial)	5.0 cc.
Urea	1.0 gm.
Chromic acid	0.5 gm.

The washing, dehydrating, and embedding were carried out in the usual manner. Sections were cut at thicknesses varying from

[3] *Mycena rubromarginata* var. **Laricis** var. nov.—Pileus 5–20 mm. latus, obtuse conicus, demum expansus et interdum late campanulatus vel convexus, glaber, striatus vel plicato-striatus, vinaceo-umbrinus, expallescens; lamellae pallidae vel cinerae, subdistantes vel distantes, adnatae, acie rubeola vel concolore; stipes pileo concolor, 2–4 cm. longus, 1–1.5 mm. crassus, glaber, fragilus; sporae 10–12 × 5–7 μ, subpyriformae; basidia 26–28 × 6–8 μ, bispora; cellulae aciei lamellarum subfusoideo-ventricosae, 40–75 × 5–12 μ. Hab. ad truncos laricinos. Specimen typicum in Herb. Mich. conservatum, A. H. Smith n. 32-229, prope Ann Arbor, Mich. June 17, 1932.

two to fifteen microns, depending on the tissue which was to be studied and the type of observation to be made.

Various stains were used, but for nuclear counts iron-alum haematoxylin with an orange G counter stain in clove oil proved to be most satisfactory. Feulgen's (18) reagent was tried on all of the species, but the resulting visibility as a rule was poor. In *Mycena capillaris,* however, it was the only stain which was reliable for nuclear counts, the others giving no differentiation whatever. Each species was a separate problem, but in general if haematoxylin was satisfactory, the other stains were also, and if the haematoxylin did not take readily, the others were equally unsatisfactory.

TAXONOMIC DATA

A careful taxonomic study has been made of all of the species in the genus which the writer has collected. In this study fruitbodies with four-spored basidia were considered typical. Two-spored forms were referred to a species only when the fruiting bodies resembled those bearing the four-spored basidia in every respect except in the number of spores born on a basidium and in the size and shape of the spores. Fruiting bodies with only four-spored basidia will be referred to hereafter as "typical forms," while those showing variation, being either two-, three- and four-spored on a single pileus, two- and three-spored, or simply two-spored, will be referred to as "variant forms." During the course of the investigation a total of ninety-five typical and variant forms were collected in Michigan; twenty-seven of these are variants. Fourteen of the variants have been correlated with typical forms, seven determined but not correlated, and six remain unidentified.

The variant forms were mostly two-spored, but in practically all a few three-spored basidia were found. In *Mycena citrinomarginata, M. dissiliens, M. leptocephala,* and *M. polygramma* var. *albida* variant forms were found with two-, three-, and four-spored basidia on a single pileus. In all cases, even on a single pileus, the spores born on two- or three-spored basidia were found to be larger than those born on four-spored basidia. The following table summarizes the change in spore size which was found to

accompany a change in the number of spores produced on a basidium. The spores of some of the species are illustrated on plate 34.

TABLE 1

COMPARISON OF SPORE-SIZE OF VARIANT AND TYPICAL FORMS OF SPECIES OF *Mycena*

Species	Variant Form [4]	Typical Form
M. alcalina	11–12 × 6.5–8 μ	8–10 × 5–6 μ
M. capillaris	11–13 × 5–6 μ	8–10 × 4–5 μ
*M. *citrinomarginata*	12–14 × 5–6 μ	9–11 × 5–6 μ
M. clavicularis	10–12 × 5–6 μ	8–10 × 4–5 μ
*M. *dissiliens*	7– 8 × 7 μ	7–8 × 5–6 μ
M. epipterygia	10–12 × 6–8 μ	9–11 × 6–7 μ
M. graveolens	8–10 × 4.5–5 μ	7–8 × 4 μ
M. immaculata	8–10 × 2.7–3 μ	7–8 × 2.5–3 μ
M. lactea	7.5–8.5 × 6–7.5 μ	7–8 × 3–4 μ
*M. *leptocephala*	11–14 × 5–6.5 μ	7–10 × 4–5 μ
M. metata	8–9 × 4–5 μ	7–8 × 3–4 μ
*M. *polygramma* var. *albida*	9–11 × 6–7 μ	8–10 × 5–6.5 μ
M. roseipallens	7–8 × 5–6 μ	6–7 × 3–4 μ
M. supina	8–11 × 7–8 μ	7–8 μ
M. viscosa	9–11 × 6.5–8 μ	8–9 × 5–6 μ

[4] The forms commonly three-spored are marked with an asterisk.

In *Mycena dissiliens* and *M. polygramma* var. *albida* there is little difference in size, and the increase is so distributed that the shape of the spore in both species is practically unaltered. In *Mycena citrinomarginata* there is a substantial increase in the length but not in width, thus accentuating the somewhat cylindrical shape of the spore. The species *M. alcalina, M. capillaris, M. clavicularis, M. graveolens, M. metata,* and *M. viscosa* all show an increase in spore size, but scarcely any change in shape. In *Mycena roseipallens, M. lactea,* and *M. supina* a change in shape has accompanied the increase in size. In the first two the change is from a more or less ellipsoid spore to one which is subglobose. In *Mycena supina* the change is from a globose spore to one which is broadly ovate. In all of the forms studied the change was constant within a species.

CYTOLOGICAL AND CULTURAL DATA

The typical forms: Nine four-spored species have been studied cytologically. These show the usual nuclear history through the

meiotic divisions, and the reorganization of the four daughter nuclei. The young basidium is characterized by the presence of two nuceli which fuse to form a large fusion nucleus. The fusion nucleus divides in the upper part of the basidium, and two reorganized daughter nuclei are regularly found. Each of these divides once, and the resulting four nuclei move toward the base of the basidium. After the spores have started to form, the four nuclei elongate and migrate upward, each into a sterigma. However, instead of migrating on into the spore as is usually the case in other agarics, each nucleus divides, one of the daughter nuclei migrating into the spore and the other eventually returning into the upper part of the basidium. Distinct division figures (PLATE 35, FIGS. 9, 27) have been found in *M. Atkinsoni* and *M. viscosa.* As a result of this division, each spore receives one nucleus and four nuclei remain in the collapsing basidium (PLATE 35, FIG. 10). These residual nuclei are constantly present in the old basidia of all of the above species except *Mycena murina,* and while third division figures have not actually been seen for all, the presence of residual nuclei in the collapsing basidia is here considered as evidence that such a division occurred.

In *Mycena murina* a striking variation from this behavior is found. In this species the third division is irregular but characteristic, and all of the daughter nuclei migrate, two into each spore. While third division figures are occasionally found with their axes arranged parallel to the longitudinal axis of the basidium (PLATE 35, FIG. 21), they are usually arranged transversely (PLATE 35, FIG. 20). As is shown (PLATE 35, FIG. 20), each of the two poles of the uppermost division figure lies beneath a sterigma. Various other stages of the division and migration of the nuclei are shown (PLATE 35, FIG. 29, 25, 26, 30, 31). All of the old basidia in this species were completely empty.

It is evident that the arrangement of the spindles in the third division may possibly have an important bearing upon the genetical identity of the nuclei in the four spores. If the heterotypic division is either the first or second division in the basidium, and if the spindles of the third division are in the sterigmata and longitudinal, one would expect two daughter nuceli, identical genetically, to migrate into a spore. On the other hand, if the spindles of the

third division are arranged transversely and daughter nuclei migrated into different sterigmata, the pairs of nuclei seen in figure 26, plate 35 might be of different genetical constitution. Since both arrangements of the third division spindles have been found in the basidia of a single pileus, this species is particularly interesting from a genetical point of view.

The variant forms (usually two-spored): The variant forms studied fall into two types; those in which the young basidium contains two primary nuclei which fuse, giving rise to a fusion nucleus, and those forms with a single nucleus in each young basidium.

The "*Mycena metata*" group: The first group of species contains *M. metata, M. clavicularis, M. graveolens, M. immaculata, M. margaritispora, M. mirata* and *M. viscosa.* The species in this group are characterized by a fusion of two primary nuclei eventually followed by three divisions in the basidium. Since this type of nuclear behavior, with the exception of the third division, is well described in the literature, it will be outlined here briefly for only one species, *Mycena metata.* The cells of the subhymenium are typically binucleate, but occasionally the larger cells are multinucleate. As the young basidium elongates the two nuclei come to lie side by side in the midportion and usually fuse before the basidium has reached its full length. Apparently the fusion takes place rather slowly. The nuclei gradually lose their outlines at the point of contact and are then distinguishable as two granular masses with a darkly staining nucleolus in each.

As soon as the fusion is complete the spireme threads appear as rather coarse, heavily staining strands (PLATE 35, FIG. 40). The fusion nucelus is 3–4 μ in diameter and one or two nucleoli can be seen within it. It gradually increases in size and moves toward the apex of the basidium. Instead of migrating directly into the apex, it comes to rest some distance below it. Judging by the frequency of such figures in all of the preparations, this "resting stage" is one of long duration. Just prior to dividing the nucleus moves farther up toward the apex and the first division follows (PLATE 35, FIG. 41). The details of the division were not worked out, but the spindle is formed within the old nuclear membrane, and a centrosome is visible at each pole. A side view

of a metaphase plate shows that at this stage the whole figure is still within the space previously occupied by the fusion nucleus. However, during the anaphase the centrosomes move back toward the walls of the basidium and at the telophase the two masses of chromatin are often found appressed against the opposite walls. The fate of the nucleoli during the division is uncertain, but they seem to be extruded into the cytoplasm where they are then indistinguishable from other granules.

The figures of the second division are very minute, but metaphase and telophase stages are frequent (PLATE 36, FIG. 1, 2). The four reorganized daughter nuclei are very small at first. They soon increase to 1.5–2 μ, develop a delicate characteristic reticulum, and migrate toward the base of the basidium. This migration carries the nuclei into the more or less constricted portion of the basidium. Just before they come to rest near the base, the sterigmata begin to develop, first as two obtuse humps, but soon elongating and assuming their characteristic narrowly conical form.

When the spores are quite large, the four nuclei situated in the lower portion of the basidium move toward the sterigmata and become elongated or beaked. The behavior of the four nuclei from the time they first become beaked to the time when the two spores are mature seems to be rather variable. As is shown in plate 36, fig. 3, the spores begin to form before the nuclei elongate. The two nuclei which lie uppermost in the basidium migrate first. Figures can be found showing the first nucleus, after it has migrated to the apex of the spore, either in a resting condition or dividing. Figures of daughter nuclei can also be found indicating that a division has been completed. The second set of nuclei may enter the spores and divide immediately (PLATE 36, FIG. 4) leaving the basidium empty, or they may divide while in the sterigmata (PLATE 36, FIG. 5). Only one of the daughter nuclei of this division then passes into the spore. The divisions in the spores and sterigmata are rather haphazard. In plate 36, fig. 4, the nucleus nearest the sterigmata is shown dividing while the apical nucleus is in a resting condition. In plate 36, fig. 5, the nucleus in one spore and those in the sterigma are shown dividing simultaneously. In plate 36, fig. 6, two nuclei in one spore are shown in the resting condition while the one nucelus in the other spore and also the nucleus just

entering the latter, are shown in practically the same stage of division. One basidium showing two resting nuclei in one spore, and two dividing nuclei in the other was also found (PLATE 36, FIG. 7).

In *Mycena margaritispora* the situation seems to be similar to that found in *Mycena metata,* the third division figures occurring rather haphazardly in both spores and sterigmata. The manner of origin of the four residual nuclei (PLATE 36, FIG. 20) has not been determined. As shown (PLATE 36, FIG. 29), in *Mycena viscosa* all four nuclei may migrate, two into each spore, and then divide simultaneously, the basidium showing no residual nuclei. However, old basidia with residual nuceli are found in this species also. In *Mycena graveolens* two residual nuclei are commonly present, occasionally four, and very seldom none at all.

The material of *M. clavicularis* and *M. immaculata* was not favorable for studying the later stages.

The "*Mycena megaspora*" group: The species of this, the second group of variant forms, are characterized by the lack of a fusion of nuclei in the basidium, the single original nucleus eventually giving rise to four daughter nuceli. Twelve species, namely *M. megaspora, M. alcalina, M. capillaris, M. cholea, M. citrinomarginata, M. dissiliens, M. lasiosperma, M. leptocephala, M. polygramma* var. *albida, M. roseipallens, M. rubromarginata* var. *Laricis,* and *M. vitilis* have been found to belong to this type. All of these are similar to the two-spored form of *Camarophyllus virgineus* studied by Bauch (1) in that the subhymenial cells and young basidia each contain a single nucleus. Because there is no fusion of nuclei in the basidium, and because the same number of chromosomes was found in the nuclei of both two- and four-spored forms, Bauch has considered the two-spored form of *C. virgineus* to be parthenogenetic. In *Mycena alcalina* which has received considerable study, the writer's observations indicate that the chromosome number, as obtained from division figures in the basidia, is apparently the same in the two-, and the four-spored forms. Since this corresponds to Bauch's findings, the writer is classifying all the forms in the "*Mycena megaspora*" group as haploid parthenogenetic forms. In the treatment which follows, *M. citrinomarginata, M. dissiliens, M. leptocephala* and *M. polygramma* var. *albida* are considered separately because they illus-

trate the nuclear behavior in parthenogenetic forms producing two, three, or four spores on a basidium.

Since *Mycena megaspora* has been studied culturally and cytologically, it will be discussed here is some detail. This species has been cultured from transfers of tissue from the pileus, groups of spores sprayed on agar plates, and from single spores isolated by the spray method (9). No clamp connections were observed in any of the cultures. The fungus grows rather slowly covering the agar in an ordinary petri dish in six to eight weeks. A 2.5 per cent malt extract agar was the most favorable medium tried, and the optimum temperature was found to be between 23 and 25 degrees centigrade. Tissue cultures grew more rapidly than the single spore cultures, but there was no difference in the type of growth produced. The hyphae grew very closely together in a compact mat along the surface of the agar and produced a fluffy, white aerial growth. The medium was penetrated to a depth of one or two millimeters by a rather sparse growth.

Fifty single spores were isolated during the course of the work, and mating experiments were carried out in an attempt to obtain diploidization. Mycelia from spores from fruit-bodies collected in different localities were used in the mating experiments, but no clamp connections were produced. Since certain Basidiomycetes (20) are known to possess the dikaryophase without showing clamp connections on the mycelium, an attempt was made to study the mycelium obtained from tissue cultures and from single spores cytologically. Due to the slow and compact manner of growth the method recommended by Sass (21) failed to give any results, and it was necessary to kill and embed mycelium grown on agar plates. Sections were cut 12 to 25 μ thick and stained with iron-alum haematoxylin or safranin and light green. While this method has obvious disadvantages, it was found to be usable. As a check, the mycelium of *Collybia clusilis* Fries, a species which produces clamp connections abundantly, was carried through the same process. Slides of *C. clusilis* showed two nuclei in each cell and abundant clamp connections while the slides of *Mycena megaspora* showed one nucleus in each cell, or occasionally two or three in very large hyphae.

In the fruit-body the cells of the stipe were found to contain

from one to six or more nuclei depending on the size of the cell. The tissue of the stipe in this species is composed of large, straight, tubular cells as well as irregular hyphae of smaller diameter which lie intertwined among the larger elements. The individual cells of the irregular hyphae were usually found with one nucleus, while the enlarged, parallel cells always contained two or more. The latter condition was also found in the enlarged cells of the pileus trama, but as a rule the cells of the gill-trama and especially those of the subhymenium were found to be uninucleate.

The single nucleus in the young basidium enlarges, keeping pace with the developing basidium (PLATE 37, FIG. 3), and finally can be found as a large and conspicuous structure just below the apex. In this stage it resembles the large fusion nucleus found in the species previously discussed. The spireme threads, however, are not coarse and their behavior prior to the single division at the apex of the basidium is not characteristic of meiosis. The large nucleus may or may not migrate up to the apex of the basidium to divide. The division figure is rather large in this species and the centrosomes are conspicuous. Here as in *Mycena metata,* the figure is formed within the old nuclear membrane. The daughter nuceli are at first very small, but enlarge rapidly as they migrate toward the base of the basidium.

The daughter nuclei stop when they have passed slightly back of the midpoint of the basidium, and at this time two sterigmata begin to develop. As the spores increase in size, the nuclei migrate upward, and as the latter approach the sterigmata they begin to elongate, assuming the beaked condition. The centrosome is visible as a darkly staining granule at the apex of the beak. One nucleus migrates into each spore. While passing through the sterigmata the fine chromatin network becomes drawn out into long strands, but is always visible in well fixed material. The two nuclei do not always migrate simultaneously but figures have been found with a nucleus present in each sterigma. As a rule, after the migration downward, one nucleus comes to rest a little below the other and remains a little behind on its migration into the spore.

Although no clearly defined division figures were found in the sterigmata, the presence of residual nuclei in the collapsing basidia

indicate that a division must have occurred. Such nuclei were usually present in *Mycena alcalina, M. cholea, M. rubromarginata,* var. *Laricis, M. roseipallens,* and *M. vitilis.* They were uncommon in *Mycena megaspora* and *M. capillaris,* and absent in *M. lasiosperma.* In *M. cholea* (PLATE 37, FIG. 16) a basidium is shown with both spores attached, each with a nucleus, and two remaining residual nuclei in the basidium. Since no basidia with four nuclei present before migration have been found in this species, the situation shown in figure 16 must have been brought about by a division when the nuclei were migrating into the spores. In *M. lasiosperma* (PLATE 37, FIG. 26) the nucleus is shown dividing after it has migrated into the spore. The migration figures (PLATE 37, FIGS. 19, 34) of *M. lasiosperma* and *M. roseipallens* are interesting. In these species the nuclear reticulum does not show any affinity for the stain and appears to be made up of a fine, hyaline, granular network. The nucleolus appears as a large opaque structure which holds the stain tenaciously, and may be mistaken for the nucleus in poorly stained preparations. During the migration of the nucleus the nucleolus becomes beaked in a characteristic manner within the nucleus and migrates within the latter into the spore. In a majority of the species studied the reticulum is characterized by the presence of darkly staining chromatin granules, and the nucleolus does not migrate into the spore as a distinct unit.

The parthenogenetic forms producing two-, three- and four-spored basidia: Since *Mycena polygramma* var. *albida* lends itself well to cytological investigation, it will be treated in detail here. The behavior of the nuclei corresponds exactly with that of the haploid parthenogenetic forms previously discussed until they are on their way into the spores. The sterigmata begin to form at about the time the two daughter nuclei cease their downward migration. As the sterigmata develop the nuclei begin to move upward toward the apex, but, after moving a short distance, each develops a long beak which stretches out, in some instances at least, almost into a filament. The apex of the beak may come to rest somewhere near the base of a sterigma or may actually enter one. Apparently the remainder of the nucleus is now drawn upward, and the beak becomes shorter and shorter. At this time

two, three, or four sterigmata are usually well developed and a spore is forming on each. When only two sterigmata are formed the nuclei do not necessarily come to rest at the apex of the basidium, but may migrate into the spore in the usual manner. Occasional residual nuclei in collapsing two-spored basidia, however, indicate that a division sometimes takes place during the migration. If three sterigmata are formed, either one of two things may happen. Occasionally the two nuclei migrate, each into a spore, leaving the third spore without a nucleus. Rather conclusive evidence of this type of behavior was found (PLATE 38, FIG. 21). In most cases, however, the three-spored basidia produce spores with one nucleus in each. This is brought about in the following manner. One nucleus rounds up near the base of a sterigma and undergoes a division, one pole lying just below one sterigma and the other pole near or under a second. As the division progresses the poles of the figure are drawn up into the sterigmata, and a U-shaped figure results (PLATE 38, FIG. 17, 18). The daughter nuclei reorganize, one in each spore. The second nucleus may also round up, but was never seen to divide in a three-spored basidium. It usually migrates just before or with the daughter nuclei resulting from the division of the first nucleus.

If the basidium produces four sterigmata, three things may happen. First, each spore may receive one nucleus. This is commonly the case and is brought about in the following manner. Each of the two nuclei which have migrated from the base of the basidium round up somewhere near the base of a sterigma. In this position each nucleus undergoes a division, the poles coming to lie under the sterigmata, and during the anaphase the unorganized nuclei begin to migrate, forming characteristic U-shaped figures. The daughter nuclei reorganize as soon as they have moved into their respective spores. The U-shaped figures were always found in pairs and there was no indication that two nuclei ever migrated into a single spore.

Secondly, one nucleus may migrate directly into one spore while the other divides in the manner described above. Thus three spores would be found with nuclei while the fourth would be without one. One basidium (PLATE 38, FIG. 29) was found indicating this.

Lastly, the two nuclei at the base of the basidium may migrate directly, one into each of two spores, thus leaving two spores without nuclei. No evidence for this type of behavior was found.

Mycena leptocephala, M. citrinomarginata, and *M. dissiliens* all exhibit this same general type of behavior. The very minute nuclei in *M. dissiliens* make it a rather unfavorable subject for study, but typical stages were found. The spores become detached from the sterigmata of *M. citrinomarginata,* and *M. leptocephala* very easily, thus making it impossible to follow the migration of the nuclei. Collection 32–597 of *M. citrinomarginata* was found to be parthenogenetic and predominantly four-spored. The nuclear behavior was similar to that described above.

GENERAL CONSIDERATIONS

In the present investigation it has been shown that in nature fruiting bodies with two-spored basidia occur along with fruitbodies bearing four-spored basidia, and that the only differences between the two are the number of spores born on a basidium and a correlated change in spore size. In certain species it has been found that these differences occur in a single pileus as well as in pilei from different localities, and at times a cytological study must be made to determine whether a form is typical or not. Such forms are clearly segregates of four-spored species. In other species the two-spored forms are more infrequent and are separated geographically from the normal form. The close similarity in all of the characters except those mentioned above, however, indicates that these also are segregates from typical forms. Thus the majority of the two-spored forms collected can reasonably be considered as segregates of the typical forms, and it is reasonable to assume that in such isolated two-spored species as *Mycena margaritispora* and *M. lasiosperma* we may be dealing with the segregated forms of otherwise unknown species. It is possible that some of these isolated two-spored species may be stable forms which have persisted while the ancestral forms have been lost in the course of evolution. It is probable that a similar relationship exists for the two-spored forms in other genera of the Agaricaceae.

As has been previously stated, the two spored character has been

used as a diagnostic character for the separation of taxonomic units. Sass (21) has suggested that the term "forma" be used "in clear cut cases." Such a designation, however, would, besides being somewhat cumbersome, give undue emphasis to the two-spored condition. For instance, if a species was found to have two-spored forms of both the "*M. metata*" and "*M. megaspora*" type, they would all be classed as *forma bispora* in spite of the difference in the behavior of the nuclei in their basidia. The writer prefers to simply speak of the segregates as forms without assigning a name to them. By so doing no implication is made concerning the relationships of one form to the other except that both are segregates of the species to which they have been assigned. In species like *M. polygramma* var. *albida* or *M. citrinomarginata* where, without a cytological examination, it is impossible to ascertain with certainty whether or not one has parthenogenetic or normal fruiting bodies, a form name would serve no purpose.

The two-spored condition also results in a change in spore size which is of importance in the differentiation of taxonomic units. The writer's observations cover a sufficiently large number of species to give a fair idea of the frequency with which the change to the two-, or three-spored condition is accompanied by an increase in spore size, the amount of this change, and its bearing on the value of spore size as a character of major taxonomic importance.

As has been shown in table 1, the larger spore size was a constant feature of the variant forms, but it seems to depend upon the species whether the shape is similar or different. Thus it is possible to predict that in the genus *Mycena,* as two-spored forms are found, the spores will measure larger than those of the typical forms. No prediction is justified, however, concerning the shape of the spore. Since this situation exists, the writer feels that the differences in the basidia and spores of variant forms, particularly in the genus *Mycena,* should not be used as a basis for establishing species. In typical forms, however, spore size and shape are remarkably constant and useful in separating species within the genus.

Since the basidia of two- and four-spored forms seem to be

approximately the same size, the question arises as to whether or not within a species, the volume of the two spores produced on a single basidium is equal to the volume of the four spores also from a single basidium. The shape of the spores of two species is such that rough approximations of their volumes can be easily made. In *M. capillaris* and *M. citrinomarginata* the spores are roughly cylindrical in shape. The following approximations may be made:

M. capillaris (4-spored) 143.3 cu. microns per spore
573 total volume of 4 spores.
M. capillaris (2-spored) 285 cu. microns per spore
570 total volume of 2 spores.
M. citrinomarginata (4-spored) 196.6 cu. microns per spore
786 cu. microns total volume of 4 spores.
M. citrinomarginata (3-spored) 255.2 cu. microns per spore
765 cu. microns total volume of 4 spores.

While only approximations, these figures indicate that there is little if any change in the volume of material used in the formation of the spores in the correlated forms, and that the difference in spore-size may possibly be explained on the basis of the amount of material available for spore formation. One would expect the above ratio to be rather constant in forms producing both two- and four-spored basidia on a single pileus. The variation in size of basidia and spores of geographical races might be sufficient to overbalance this difference if the two-spored form from one locality is compared with a four-spored form from a different locality.

In the species of *Mycena* investigated in this study the basidia were always emptied of protoplasm before the spores were shed. If this does not happen, however, it is doubtful if there would be an increase in spore size to the extent described above. In other genera the basidia of the variant forms are not always emptied of protoplasm as completely as in *Mycena* and in such cases it is not likely that an equal increase in spore size would be found in the variant forms. *Naucoria semiorbicularis* f. *bispora* Sass (21) seems to be an example.

CYTOLOGICAL CONSIDERATIONS

The typical forms: The nuclear behavior in eight of the four-spored species agrees with that found in *M. galericulata* as described by Maire (17) except that the spores are usually uninucleate. The occasional binucleate spores which were found may have obtained their nuclei either by the division of the nucleus after entering the spore, or by the migration into the spore of the daughter nuclei resulting from a third division.

Wager (22) figured two reorganized nuclei resulting from the first division in *Mycena galericulata*. Wakayama (23) in his studies of a large number of agarics in various genera found that in *Mycena haematopoda* the daughter nuclei of the first division always reorganize before undergoing the second. In species of other genera, he found no period of interkinesis. All of the forms studied by the writer exhibiting meiosis also show a similar short period of interkinesis between the first and second divisions.

Evidence of the regular occurrence of a third division in the basidium has been found in all of the typically four-spored species investigated and may well be considered typical for the genus. It has been shown that this division is postponed until the nuclei are starting to migrate or are actually migrating into the spores. It is thus definitely removed from the meiotic divisions in time as well as location. In the species of *Craterellus* and *Clavaria* which have been studied (8) the third division may either follow soon after the first two, and before the nuclei have begun to migrate, or take place in the basidium after the spores are partly formed.

Two-spored forms of the *Mycena metata* group: The third division which was found to be characteristic of the four-spored forms is also present in the group of two-spored forms characterized by *Mycena metata*. In this group we find a situation essentially similar to that found by Sass (21) in the two-spored form of *Coprinus ephemerus*. Two nuclei regularly migrate into each spore. Because attempts to germinate the spores in this group of species have been unsatisfactory, it has been impossible to determine whether they are "heterothallic" or "homothallic." Since their nuclear history is essentially like that of the two-spored form of *C. ephemerus*, however, one would expect them to be "homothallic."

In this group the fusion nucleus gives rise to four daughter nuclei. One of the daughter nuclei migrates into each spore where it divides. The remaining two nuclei may divide in the sterigmata or after entering their respective spores. If the division takes place before entrance, one would expect to find residual nuclei in the basidium. If it takes place after the nucleus enters the spore, the basidium should be empty. Division figures have been found in the spores and sterigmata of most of the species in this group. Both empty basidia and those with residual nuclei have also been found.

Since both Sass and Buhr have interpreted the residual nuclei which they observed in the collapsing basidia of the forms they studied as remains of two of the original four nuclei produced by the two meiotic divisions, their descriptions and figures should be reconsidered in the light of the above facts. In the two species of *Mycena* which Buhr studied, the residual nuclei could have originated in either manner. The basidia shown in his figures do not have the spores attached. In *Nolanea cetrata,* however, his illustrations are convincing enough. The attached spores (FIG. 11) each have a nucleus and two have remained in the basidium. In *Psalliota campestris* (FIG. 24) the residual nuclei show their usual structure as do those of *Pholiota erebia* (FIG. 4). Sass (21), plate 66, figures 60 and 61 of *Naucoria semiorbicularis,* illustrates the residual nuclei as possessing their normal structure, while on plate 67, fig. 73 the one residual nucleus shown for *Galera tenera* is opaque.

In all of the writer's slides the residual nuclei stained very darkly, similar to the telophases of the earlier divisions, and were never found to reorganize sufficiently to show either a reticulum or nucleolus. Buhr's (4) figures 36 and 37 of *Mycena debilis* certainly illustrate a condition closely approaching this. It is quite possible that the residual nuclei originate differently in different species or genera. Certainly if two of the original four nuclei disintegrate, one would expect to find early stages in which the structure of the nucleus was still visible. If, on the other hand, the nuclei left in the basidium by the third division degenerate without reorganizing, no such early stages could be expected.

The omission of a division in the parthenogenetic forms studied

by the writer is interesting because it furnishes still more evidence in support of the statement that three divisions are characteristic in the basidia of this genus. In both the typical forms and those of the *M. metata* type two divisions in the apex of the basidium are characteristic before the sterigmata begin to form. In the parthenogenetic forms only one such division occurs. Thus it is reasonable to assume that the reduction divisions are omitted and in their place a single mitosis occurs. This is apparently the same situation which Bauch (1) disscovered in *Camarophyllus virgineus*. In the two-spored form of that species Bauch found that one of the two nuclei resulting from the single division at the apex of the basidium migrated into each spore. In the parthenogenetic forms studied by the writer two divisions were characteristic, the second, however, corresponded closely in both time and irregularity of occurrence to the third division found in the typical forms and in the variants of the *M. metata* type. It is no doubt homologous with it.

The manner in which the last division functions in the distribution of nuclei to the various spores of the forms producing two, three and four sterigmata is interesting. As a result of it, it is possible to have four-spored forms in the genus *Mycena* which are indistinguishable unless the material is examined cytologically. Such forms have been collected. No particular significance can be attached to the spores which do not receive nuclei, but it would be interesting to determine their approximate percentage and whether or not they are discharged from the basidium in the usual way. Since two-spored forms of both cytological types are common in a single genus it is apparent that the nuclear history is not necessarily concerned with the number of sterigmata produced on a basidium. In the *M. metata* type the behavior of the nuclei is typical, but the basidia are constantly two-spored. In the *M. megaspora* type the nuclear behavior varies but the number of sterigmata may be four. That the two-spored condition is a constant genetical character in the forms of the *M. metata* type is borne out to some extent at least by the fact that the forms of this group can be collected year after year in the same as well as in widely scattered localities under a variety of weather conditions. The parthenogenetic series, however, does not present a clear cut

case because of the numerous specimens producing two, three, and four spores on different basidia in a single pileus.

The results of the present investigation when considered in the light of previously known facts indicate that in a closely related group as well as in a group of distantly related forms, the same types of variation in the nuclear behavior are encountered. Buhr (4), summarizing the information concerning two-spored forms in the Hymenomycetes, lists sixteen species having a normal fusion with the customary two divisions following (except in the species of *Clavaria* and *Craterellus* which have a third). In contrast to this, only two are listed definitely as being parthenogenetic. He includes two species of *Mycena* in the first group and one in the second. The results of this investigation add seven species of *Mycena* to the former group, twelve to the latter, and prove conclusively that the parthenogenetic type of variant is common in the genus.

The forms and species showing a reduction in the number of spores born on the basidium presents an interesting series in the Hymenomycetes. Species of Clavariaceae and Cantharellae are known to have basidia bearing more than four spores and also to have two-spored forms. In the Agaricaceae, four sterigmata are usually formed. Parthenogenetic four-spored forms as well as those varying between the two- and four-spored condition are known in *Mycena.* In *Galera,* Bresadola (3) has figured a form of *G. silignea* with monosporous basidia. In *Naucoria lenticeps* Peck, fruiting bodies bearing basidia with one, two, three or four sterigmata have been found by the writer. Thus within a single species or in the group as a whole variations of from one to four spores are known to occur.

The presence of a third division in most of the basidia of typical and variant forms of *Mycena* supports the hypothesis that the species of this genus are rather closely related to primitive types which are characterized by three successive nuclear divisions in the basidium. The variations found indicate that the character is in a transitional state.

SUMMARY

1. During the course of the present investigation sixty-five typical and twenty-seven variant forms were collected.

2. One species *Mycena cholea* and one variety *Mycena rubromarginata* var. *Laricis* are described as new.

3. Of the twenty-seven variant forms collected, fourteen have been correlated with typical forms.

4. An increase in spore size has been found to accompany the change to the two- or three-spored condition. A change in spore shape may or may not accompany the change in the number of spores born on a basidium.

5. The writer believes that the variations in spore size and shape noted for the variant forms should not be used as basic distinctions in describing new species.

6. Nine typically four-spored forms have been studied cytologically. In eight, *M. Atkinsoni, M. alcalina, M. hemisphaerica, M. haematopoda, M. leptocephala, M. sanguinolenta, M. stannea* and *M. viscosa* the nuclear history is characterized by a fusion of two primary nuclei followed by two meiotic divisions, and a third division before the nuclei migrate into the spores. Four nuclei usually degenerate in each basidium. In *Mycena murina* there is a similar third division, but each spore receives two nuclei.

7. Of the variant forms studied, *M. metata, M. clavicularis, M. graveolens, M. immaculata, M. margaritispora, M. mirata,* and *M. viscosa* all possess two primary nuclei which fuse. Three divisions were found to be characteristic, but the third was rather haphazard, occurring either in the spores or basidia. *M. megaspora, M. alcalina, M. capillaris, M. cholea, M. citrinomarginata, M. dissiliens, M. lasiosperma, M. leptocephala, M. polygramma* var. *albida, M. roseipallens, M. rubromarginata* var. *Laricis,* and *M. vitilis* were found to have a single nucleus in the young basidium and are considered to be parthenogenetic. Instead of the usual three divisions found in the other forms in this genus, the parthenogenetic forms were characterized by two, the last of which is apparently homologous with the third division found in the other types.

8. Four of the parthenogenetic forms, *M. citrinomarginata, M. dissiliens, M. leptocephala* and *M. polygramma* var. *albida* were found to produce two, three, or four spores on different basidia of a single pileus. In these forms the transverse arrangement of the spindles in the second division was found to form a mechanism whereby a nucleus was usually distributed to each spore. The

process, however, was found to be rather variable and spores without nuclei were found. The latter condition may be brought about by a nucleus failing to undergo the last division before migration, or possibly by the longitudinal instead of the transverse arrangement of the second division spindle.

9. Since both the *M. metata* and *M. megaspora* types of variants are common in *Mycena,* it seems improbable that the nuclear behavior in the basidium is in any way connected with the number of spores produced on a basidium.

EXPLANATION OF PLATES

PLATE 34

The drawings were made with the aid of a camera lucida using a Bausch & Lomb 3 mm. N. A. 0.85 objective and a 25 × ocular. As reproduced, the magnification is approximately 830 ×.

Fig. 1, Spores of four-spored form of *Mycena epipterygia.*
Fig. 2, Spores of two-spored form of *Mycena epipterygia.*
Fig. 3, Spores of two-spored form of *Mycena alcalina.*
Fig. 7, Spores of four-spored form of *Mycena alcalina.*
Fig. 4, Spores of two-spored form of *Mycena capillaris.*
Fig. 5, Spores of four-spored form of *Mycena capillaris.*
Fig. 6, Spores of two-spored form of *Mycena roseipallens.*
Fig. 9, Spores of four-spored form of *Mycena roseipallens.*
Fig. 8, Spores of four-spored form of *Mycena viscosa.*
Fig. 11, Spores of two-spored form of *Mycena viscosa.*
Fig. 10, Spores of two-spored form of *Mycena lactea.*
Fig. 13, Spores of four-spored form of *Mycena lactea.*
Fig. 12, Spores of four-spored form of *Mycena immaculata.*
Fig. 14, Spores of two-spored form of *Mycena immaculata.*
Fig. 15, Spores of four-spored form of *Mycena metata.*
Fig. 16, Spores of two-spored form of *Mycena metata.*
Fig. 17, Spores of three-spored form of *Mycena citrinomarginata.*
Fig. 18, Spores of four-spored form of *Mycena citrinomarginata.*
Fig. 19, Spores of two-spored form of *Mycena mirata.*

PLATES 35–38 INC.

The drawings were made with the aid of a camera lucida using a Zeiss 2 mm. N. A. 1.3 apochromatic objective and a 20 × compensating ocular. All are reproduced at a magnification of approximately 1100 ×.

PLATE 35

Fig. 1–10 inc. *Mycena Atkinsoni.* 1, a young basidium showing two primary nuclei; 2, the fusion nucleus; 3, a metaphase stage of the first division; 4, reorganized nuclei after first division; 5, telophase of second division; 6,

four reorganized daughter nuclei; 7, four nuclei near the center of the basidium before the spores have begun to form; 8, four spores, each with a nucleus, and four residual nuclei in the basidium; 9, telophase of third division; 10, old basidium with four residual nuclei.

Fig. 11–26 and 29–32 inc. *Mycena murina.* 11, young basidium with two nuclei; 12, fusion nucleus; 13, first division; 14, two of the four daughter nuclei which have migrated up from near the base of the basidium in preparation for the third division; 15, four daughter nuclei in the upper part of the basidium a short time after the second division; 16, four nuclei in the lower part of the basidium; 17, telophase stages of the third division, the spores were no doubt broken off; 18, two reorganized nuclei after the first division; 19, telophase stages of second division; 20, metaphase stages of third division, spindles arranged transversely; 21, metaphase stages of third division, spindles arranged longitudinally; 22, three spores with one nucleus each and one nucleus in each of the four sterigmata; 23, metaphase figure of third division, spindle arranged transversely; 24, telophase of third division; 25, one nucleus about to enter a spore and a second dividing at the base of the sterigma; 26, two nuclei in each of four sterigmata; 29, three spores each with a nucleus, and a second nucleus about to enter each spore; 30 and 31, the same basidium, each of the four spores contains two nuclei and the basidium is empty; 32, portion of a basidium showing one spore with a nucleus and a second nucleus dividing transversely at the base of the sterigma (32 and 24 are of the same basidium but at different focal planes).

Fig. 27, 28 and 35, *Mycena viscosa,* four-spored. 27, metaphase of third division, the spores have probably been broken off; 28, telophase of third division; 35, three spores with nuclei, four residual nuclei in the basidium.

Fig. 33, 34, 36, 37, 38, *Mycena hemisphaerica.* 33, third division figures; 34, spores with dividing nuclei, the spores had been broken from their attachment; 36, basidium with four spores each with a nucleus, four residual nuclei in the basidium; 37 and 38, four residual nuclei in each of two old basidia.

Fig. 39–43 inc. *Mycena metata,* two-spored. 39, young basidium with two primary nuclei; 40, fusion nucleus; 41, anaphase of first division; 42, telophase of first division; 43, reorganized nuclei from first division.

PLATE 36

Fig. 1–10 and 12, *Mycena metata,* two-spored. 1, metaphase of second division; 2, telophase of second division; 3, a basidium with four nuclei, two spores are shown in an early stage of development; 4, a basidium with no residual nuclei, one nucleus at the base on one spore shown dividing while the nucleus at the apex is in the resting condition, in the other spore the nucleus at the apex is dividing, it can not be definitely determined whether the nucleus at the base of the second spore had just entered or whether it also was dividing; 5, two nuclei in the sterigmata and one in a spore dividing simultaneously; 6, one nucleus dividing in a sterigma and one in the apex of the spore, in the second spore both nuclei are in the resting state; 7, two nuclei dividing in one spore, two resting nuclei in the other, there are no residual nuclei in the basidium; 8, one spore with three nuclei, one spore with one nucleus (in the telophase stage) and two nuclei in the basidium; 9, a spore with

four nuclei; 10, a spore with two nuclei dividing and no residual nuclei in the basidium; 12, a collapsing basidium with two residual nuclei.

Fig. 13–15, *Mycena stannea,* four-spored. 13, four nuclei beaked, the centrosome is at the apex of the beak; 14, daughter nuclei of the third division; 15, a portion of an old basidium showing two spores, the small basidium shows two primary nuclei.

Fig. 11, 16–21 and 23, *Mycena margaritispora.* 11, a spore with four nuclei; 16, a basidium with two primary nuclei; 17, first division, spindle arranged transversely; 18, second division; 19, third division (in spores and sterigmata); 20, four residual nuclei in an old basidium; 21, two residual nuclei in an old basidium; 23, first division, spindle arranged longitudinally.

Fig. 24–27, and 29, *Mycena viscosa,* two-spored. 24, basidium with two primary nuclei; 25, second division, one spindle longitudinal and the other nearly transverse and viewed from the metaphase plate; 26, four nuclei in the lower portion of the basidium; 27, nuclei elongating preparatory to passing through the sterigmata; 29, an empty basidium with two spores each with two nuclei (all of the nuclei are seen to be practically in the same stage of division).

Fig. 22, *Mycena sanguinolenta,* an old basidium showing residual nuclei and two spores each with a nucleus. Two sterigmata were apparently removed in sectioning.

Fig. 30, 31, 38, and 39, *Mycena mirata.* 30, young basidium with two primary nuclei; 31, four nuclei in a basidium at the time the sterigmata are forming; 38 and 39, old basidia, one showing residual nuclei.

Fig. 35 and 36, *Mycena clavicularis,* two-spored. 35, young basidium with primary nuclei; 36, basidium with four nuclei shortly after the second division.

Fig. 37 and 44, *Mycena immaculata,* two-spored. 37, basidium with two primary nuclei; 44, four nuclei at about the time the spores start to form.

Fig. 32, 33, 40–43, *Mycena graveolens,* two-spored. 32, young basidium with two primary nuclei; 33, a spore with four nuclei; 40, basidium with four nuclei; 41, a basidium with two spores attached, each with three nuclei (it is possible that one nucleus divided while in the sterigma and that the other migrated without dividing. The pairs of nuclei at the apex are interpreted as daughter nuclei resulting from a division in the spore); 42, an old basidium with two residual nuclei; 43, an old basidium with four residual nuclei.

PLATE 37

Fig. 1–10 inc. *Mycena megaspora.* 1, young basidium with a single nucleus; 2, young basidium and subhymenial cells with one nucleus each; 3, a developing basidium with its enlarging nucleus; 4, a metaphase stage of the only division which takes place near the apex of the basidium before the sterigmata begin to form; 5, telophase stage; 6, the enlarging basidium with the two reorganized nuclei; 7, an early stage in the formation of the sterigmata; 8, the two nuclei in the basidium have started to migrate, the spores are still very small; 9, a nucleus partly in the spore and partly in the sterigma; 10, one nucleus is seen in each spore, the basidium contains no residual nuclei.

Fig. 11, 12 and 19, *Mycena roscipallens.* 11, the young basidium with its single nucleus; 12, and 19, the nuclei are shown partly elongated, the nucleolus is also shown migrating as a distinct body within the nucleus.

Fig. 13 and 14, *Mycena rubromarginata* var. *Laricis.* 13, young basidium and basal cell each with one nucleus; 14, two nuclei each migrating into its respective spore.

Fig. 16, 17, 23, *Mycena cholea.* 23, young basidium with a single nucleus; 17, basidium with two nuclei at about the time that the spores begin to form; 16, old basidium with two spores attached, each with a nucleus and two residual nuclei in the basidium indicating that a division must have taken place during migration.

Fig. 15, 22, *Mycena capillaris,* two-spored. 15, the nuclei are elongating preparatory to passing through the sterigmata; 22, young basidium with a single nucleus.

Fig. 20, 21, 26, 27, and 34, *Mycena lasiosperma.* 20, young basidium with a single nucleus; 21, division at apex of basidium; 26, spore showing nucleus in telophase stage; 27, portion of a basidium showing how the nucleolus elongates with the nucleus; 34, nuclei elongating preparatory to entering the spores.

Fig. 18, 24, *Mycena vitilis.* 18, basidium with spore beginning to form; 24, young basidium with a single nucleus.

Fig. 28, 30, 31, 36–38, *Mycena leptocephala,* parthenogenetic form. 28, young basidia and subhymenial cell showing a single nucleus each; 30, a basidium with two sterigmata beginning to form and two nuclei slightly elongated; 31, nucleus dividing at base of sterigmata; 36, young basidium with a single nucleus; 37, the enlarged nucleus prior to dividing; 38, four sterigmata forming on one basidium and only two nuclei in the basidium.

Fig. 35, 42, 43, *Mycena citrinomarginata,* parthenogenetic form. 35, nuclear division at the base of the sterigmata; 42, nuclear division at the apex of the basidium before the spores have begun to form; 43, a basidium with two nuclei and two sterigmata.

Fig. 32, 33, 39, 40, 41, *Mycena dissiliens,* parthenogenetic form. 32, young basidium and basal cell each with a single nucleus; 33, the single division at the apex of the basidium; 39, the two daughter nuclei after they have moved downward; 40, the nuclei near the base of the basidium becoming beaked (note the three sterigmata which are nearly formed); 41, telophase of the division at the base of the sterigma.

PLATE 38

Fig. 1–29, *Mycena polygramma* var. *albida,* parthenogenetic form. All of the figures were drawn from the basidia of a single pileus. 1–7, illustrate the usual type of development in parthenogenetic forms; 8–10, illustrate basidia each with two nuclei, but two, three, and four sterigmata; 11–13, show nuclear elongation in two-spored basidia; 14, the two nuclei have assumed a position which indicates that migration has ceased temporarily (the stage shown in fig. 11 precedes this one); 15–18, portions of basidia showing the division at the bases of the sterigmata; 19–20, nuclear migration in two-spored basidia; 21, a basidium which produced three spores, but in which

both nuclei migrated without dividing thus one spore did not receive a nucleus; *22*, a basidium showing two nearly mature spores and one partly developed, the origin of the single nucleus in the basidium can not be definitely determined; *23–24*, three-spored basidia, each spore receiving a nucleus; *25*, a three-spored basidium; each spore with a nucleus; *26*, two nuclei in a basidium and four spores forming; *27*, each nucleus divides, and thus each spore receives a nucleus; *28*, a basidium with four mature spores, each with a nucleus; *29*, a four-spored basidium in which only three spores received nuclei.

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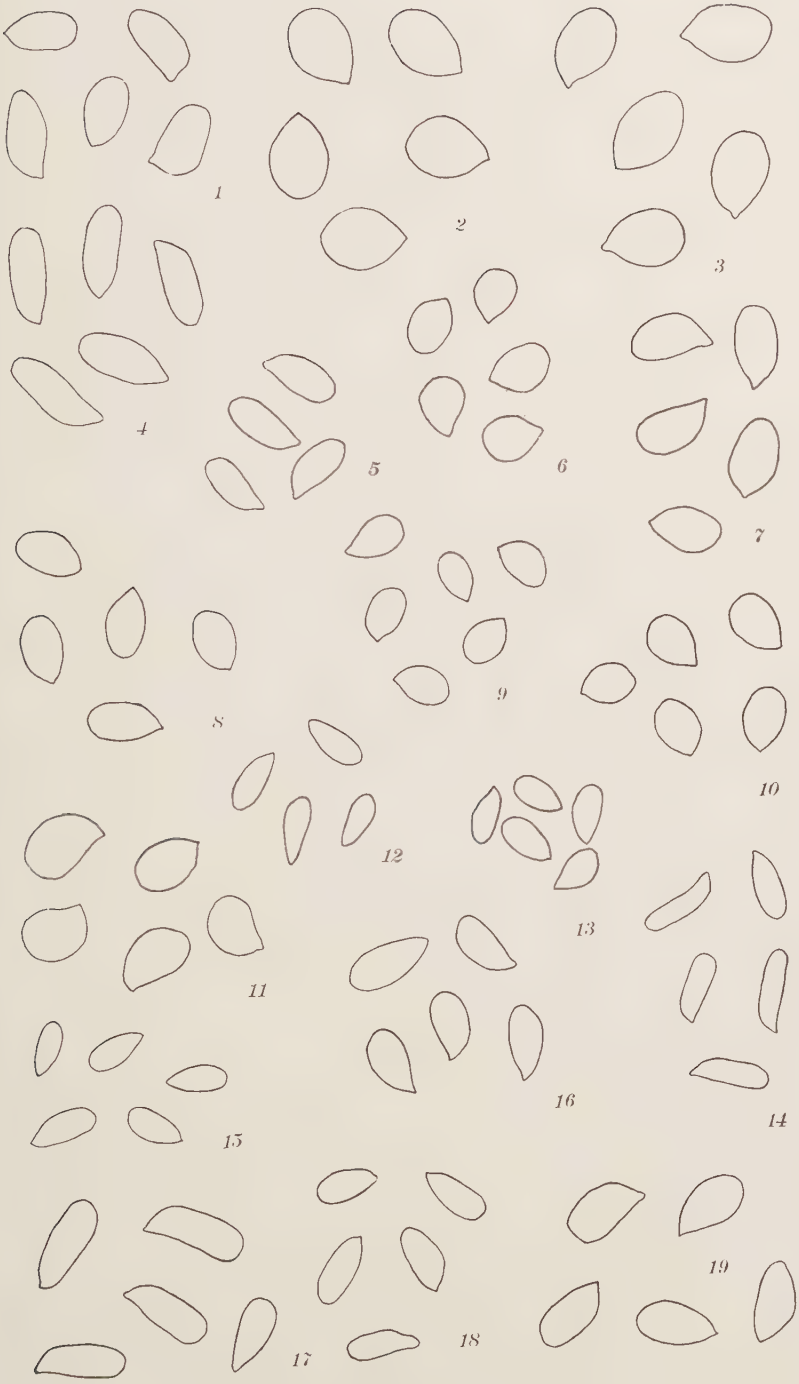

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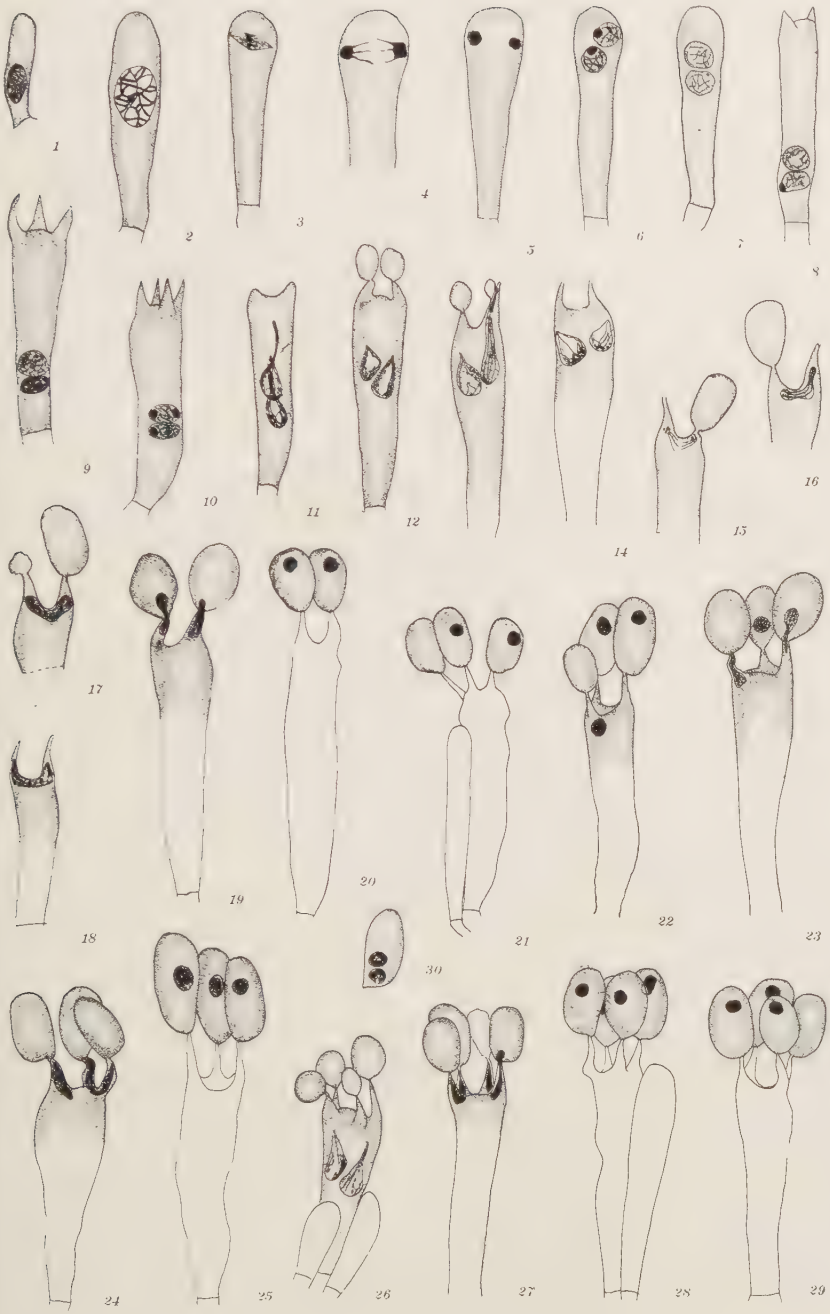

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